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Gas Chromatography-Mass Spectrometry Based Metabolite Profiling and *In Silico* Activity Prediction of Phytoconstituents in *Phoenix dactylifera L.* Leaves Extracts of Kachchh Region

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Abstract

Phoenix dactylifera L. commonly known as date palm is one of the major flora grown in the Kachchh region. Date palm is dioecious plant, that is, it owns separate male and female trees. Gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography, high-performance thin layer chromatography, and infrared red spectroscopy are some widely utilized sophisticated analytical techniques for the identification of bioactive phytoconstituents in medicinal plants. The present study aims to identify phytoconstituents present in ethyl acetate extract of male and female date palm leaves using GC-MS. These identified bioactive phytoconstituents were subjected to *in silico* useful biological activity prediction using pass online server. The study concluded that both male and female extracts were enriched phytochemicals possessing diverse valuable biological activity such as antihypercholestrolemic, anticancer, antineopolastic, lipid metabolism regulator, hepatoprotectant, and apoptosis antagonist.

Keywords: Alpha amyrin, Beta-sitosterol, Gas chromatography, Pass online, *Phoenix dactylifera* L.

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Introduction

Plants are indispensible provenance of medicine since ancient times as they are comprised of chemical entities having relevant biological and pharmacological traits.[1,2] Plant-based conventional therapeutics are preferentially utilized over contemporary synthetic medications because of their environmentally compatible characteristics and they are extinct of side effects.[3] Phytochemicals present in plants possess diverse range of beneficial biological activities such as antioxidant, anticancer, analgesic, anti-microbial, anti-diarrheal, and so on.[4] Gas chromatography-mass spectrometry (GC-MS) is a widely used hyphenated analytical technique for analysis of biologically active phyto-constituents such as fatty acids, lipids, steroids, essential oil, alkaloids, and terpenoids.[5,6]

Phoenix dactylifera L. commonly known as date palm is dioecious plant which means it owns individual male and female flora.[7] *P. dactylifera L*. fruit extracts are enriched with phytochemicals possessing numerous health boosting effects such as antioxidant, free radical scavenging, coronary heart disorder prevention, anti-cancer, hepatoprotective, and anti-inflammatory traits.[8] A topical cream derived from *P. dactylifera L*. fruits exert anti-aging, anti-acne, moisturizing, and whitening effects on healthy skin.[9] *P. dactylifera L*. leaf and stem extracts exert advantageous effect in regulation of lipid profiles and thyroid hormones.[10] Date palm cultivation preoccupies 16000 km^2 land of Kachchh region. Date palm fruits are sold at the price ranging from 10 to 400 INR or more. However, date palm leaves find no discrete usage.[11] The present study includes *in silico* biological activity prediction of different phytoconstituents identified by GC-MS analysis of male and female date palm leaves extracts of Kachchh region.

Materials and Methods

Sample Collection and Storage

Male and female *P. dactylifera L*. leaves were collected from local farm house at Bhuj during the month of December. The leaves were cleaned with distilled water and dried under shade. The resultant leaves were utilized for further studies.

Extract Preparation

Male and female date palm leaves were powdered using mechanical grinder. For the preparation of extracts, 10 g of powdered leaves sample were extracted with 100 ml of ethyl acetate by maceration for 24 h. The resultant extracts were diluted to appropriate concentration for GC-MS analysis.

GC-MS Analysis

GC-MS analysis was carried using Shimadzu GC-MS QP 2010. Helium was used as a carrier gas with constant flow rate of 1.50 ml/min. Sample volume of 2 µL was injected into the column and at the pressure 88.6 kPa. The final column oven temperature was set to 280°C with the heating rate 10°C/min. The mass spectrum of each component was recorded between the mass range m/z 50 and m/z 750. Name, molecular weight, and molecular structure of each detected compounds were done

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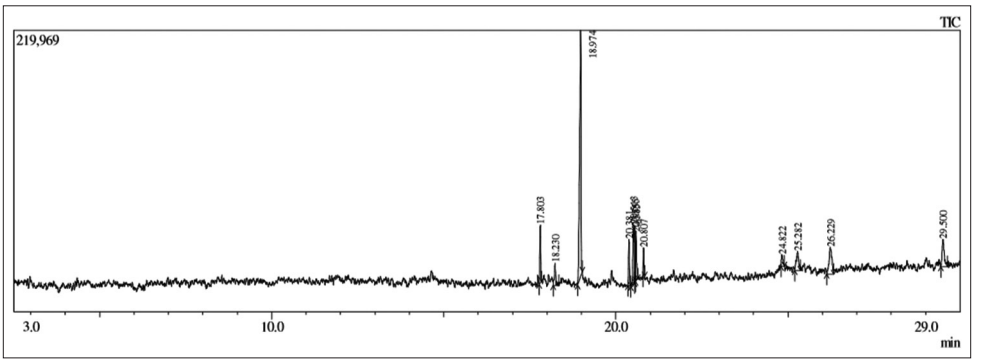

Figure 1: Gas chromatogram of phytoconstituents of female date palm ethyl acetate extract

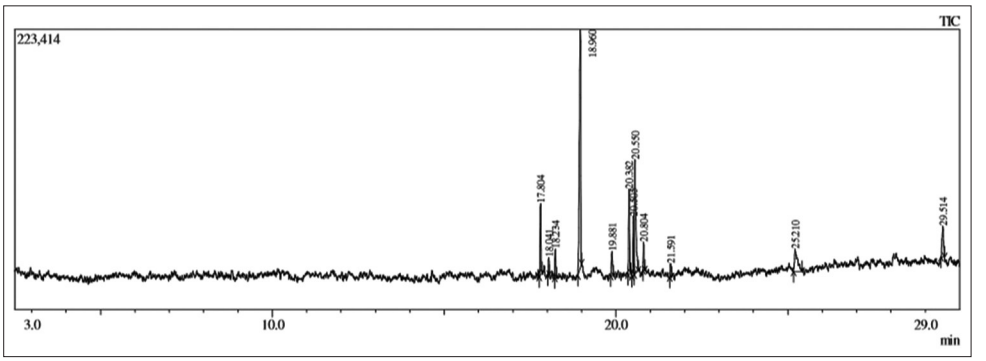

Figure 2: Gas chromatogram of phytoconstituents of male date palm ethyl acetate extract

Table 1: Retention time, relative percentage, and name of phytoconstituents identified using NIST and WILEY library from Gas Chromatogram in female date palm ethyl acetate leaves extract

S. No.	*Retention time*	*Relative percentage*	*Compound name*
1.	17.80	5.50	Neophytadiene
2.	18.23	3.50	Phytol acetate
3.	18.97	40.05	n-hexadecanoic acid
4.	20.38	4.74	phytol
5.	20.51	8.83	8-Dodecen-1-ol, acetate
6.	20.55	5.98	3-6-Nonadien-1-yl-acetate
7.	20.58	5.16	Octadec-9-enoic acid
8.	20.80	2.61	Octadecanoic acid
9.	24.82	2.11	Beta-sitosterol
10.	25.28	5.17	Alpha-amyrin
11.	26.23	9.18	Lupeol
12.	29.500	7.17	Hexacosane

Table 2: Retention time, relative percentage, and name of phytoconstituents identified using NIST and WILEY library from Gas Chromatogram in male date palm ethyl acetate leaves extract

S. No.	*Retention time*	*Relative percentage*	*Compound name*
1.	17.80	6.67	Neophytadiene
2.	18.04	1.40	Phytol acetate
3.	18.23	2.53	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
4.	18.96	34.42	n-hexadecanoic acid
5.	19.88	3.21	Heptadecanoic acid
6.	20.38	8.26	Phytol
7.	20.50	7.71	cis-7-Dodecen-1-yl acetate
8.	20.55	18.57	9,12,15-Octadecatrienoic acid
9.	20.80	2.45	Octadecanoic acid
10.	21.59	1.62	Paramethaqualone
11.	25.21	8.05	Quinazolin-4(3H)-one
12.	29.51	5.10	Hexacosane

by comparing mass spectra of unknown compound with mass spectra of standard compound found in database of NIST 11, NIST 11s, and Wiley library.

Figures 1 and 2 represent total ion chromatogram of GC-MS analysis of female and male date palm leaves extracts respectively.

In Silico Activity Prediction

In silico activity prediction of selected phytoconstituents identified by GC-MS was done using Pass online server (www.way2drug.com).[12,13] It reveals the prediction score for biological traits by means of ratio of probability to be active Pa to the probability to be inactive Pi. Enhanced value of Pa indicates higher probability of a given biological activity for the component.

Results and Discussion

Neophytadiene is a triterpenoid compound possessing antifungal, anti-oxidant, anti-microbial, anti-inflammatory, anti-pyretic, and analgesic properties.[14,15] It also aids healing of headache, rheumatism, and some skin ailments.[16] Phytol possess anti-noconceptive, anti-oxidant, anti-microbial, anti-cancer, anxiolytic, anti-depressant, anti-inflammatory, anti-hyperalgesic,

Table 3: Pa and Pi values corresponding to useful biological activity of phytoconstituents of date palm leaves extracts evaluated using pass online

S. No.	*Compound name*	*Activity*	*Pa*	*Pi*
1.	Neophytadiene	Phobic disorders treatment	0.86	0.014
		Testosterone 17 beta-dehydrogenase (NADP+) inhibitor	0.853	0.014
		Saccharopepsin inhibitor	0.833	0.014
		Acrocylindropepsin inhibitor	0.833	0.014
		Chymosin inhibitor	0.833	0.014
2.	n- hexadecanoic acid	Acylcarnitine hydrolase inhibitor	0.973	0.001
		Alkylacetylglycerophosphatase inhibitor	0.966	0.001
		Antieczematic	0.920	0.004
		Sugar-phosphatase inhibitor	0.945	0.002
		Chymosin inhibitor	0.961	0.002
3.	Phytol	Prenyl-diphosphatase inhibitor	0.911	0.002
		Retinol dehydrogenase inhibitor	0.907	0.001
		Ubiquinol-cytochrome-c reductase inhibitor	0.905	0.005
		Lipid metabolism regultor	0.828	0.005
		Carboxypeptidase Taq inhibitor	0.750	0.012
4.	8-Dodecen-1-ol, acetate	Mucomembranous protector	0.921	0.004
		Antieczematic	0.916	0.004
		Lipid metabolism regulator	0.906	0.004
		Acylcarnitine hydrolase inhibitor	0.829	0.010
		Antihypercholesterolemic	0.789	0.005
5.	3-6-Nonadien-1-yl-acetate	Antieczematic	0.935	0.003
		Lipid metabolism regulator	0.881	0.004
		Anti-inflammatory	0.859	0.005
		Phobic disorders treatment	0.858	0.015
		Saccharopepsin inhibitor	0.839	0.013
6.	octadec-9-enoic acid	Acylcarnitine hydrolase inhibitor	0.956	0.002
		Antieczematic	0.947	0.003
		Lipid metabolism regulator	0.908	0.004
		Vasoprotector	0.872	0.003
		Antimutagenic	0.852	0.003
7.	Octadecanoic acid	Acylcarnitine hydrolase inhibitor	0.973	0.001
		Levanase inhibitor	0.931	0.002
		Antieczematic	0.920	0.004
		Urethanase inhibitor	0.864	0.003
		Antiseborrheic	0.866	0.008
8.	Beta-sitosterol	Antihypercholesterolemic	0.960	0.002
		Cholesterol antagonist	0.957	0.001
		Acylcarnitine hydrolase inhibitor	0.928	0.003
		Hypolipemic	0.924	0.004
		Chemopreventive	0.831	0.003
9.	Alpha-amyrin	Insulin promoter	0.934	0.002
		Hepatoprotectant	0.926	0.002
		Apoptosis agonist	0.911	0.004
		Antineopolastic	0.901	0.005
		Anti-inflammatory	0.889	0.004
10.	Lupeol	Antineoplastic	0.950	0.004
		Hepatoprotectant	0.907	0.002
		Apoptosis agonist	0.883	0.005
		Acylcarnitine hydrolase inhibitor	0.869	0.006
		Antiprotozoal	0.891	0.003

anti-pyretic, and anti-arthritic activities.[17,18] Phytol is used as incense substance and is incorporated in variety of cosmetic and non-cosmetic formulations.[19] Phospholipase A2 promotes the formation of lipid intermediates of inflammation by stimulating hydrolysis of membrane phospholipids. Hexadecanoic acid exerts anti-inflammatory action by inhibiting phospholipase A2.[20,21] Hexadecanoic acid also possess anti-bacterial, anti-fungal, anti-oxidant, anti-psychotic, and anti-androgenic.[22,23] Octadecanoic acid is anti-bacterial, anti-fungal, and anti-tumor activities.[24] It is employed as solidifying agent in cosmetic formulations.[25] Oleic acid stimulates insulin signaling by retarding protein tyrosine phosphatase 1B and leptin signaling pathway.[26] B-sitosterol is a bioactive phytosterol possessing anti-oxidant, anti-diabetic, immunomodulatory, anti-microbial, anti-cancer, lipid lowering, hepatoprotective, wound healing, anti-inflammatory, antixiolytic, and sedative properties.[27] α-amyrin possess anti-convulsant, anxiolytic, anti-depressant, anti-inflammatory, analgesic, gastro protective, hepatoprotective, anti-pancreatitis, anti-colititis, anti-hyperglycemic, and hypolipidemic traits.[28] Lupeol is lupane type triterpenoid having wound healing, anti-cancer, anti-mutagenic, anti-protozoal, chemopreventive, anti-inflammatory, cardioprotective, hepatoprotective,

anti-microbial, anti-allergic, and anti-urolithiatic properties.[29,30] Z-8-dodecen-1-ol acetate is a sex pheromone of oriental fruit moth (*Grapholita molesta*).[31]

N-hexadecanoic acid, octadecanoic acid, octadec-9-enoic acid, and beta sitosterol were significantly predicted as potent acylcarnitine hydrolase inhibitors (Pa > 0.9). Acylcarnitines stimulate the balance of intracellular sugar and lipid metabolism, peroxidation of fatty acids, synthesis of ketone bodies, and metabolism of amino acids.[32] Lipid metabolism is concerned with production of variety of structural and functional lipids and their deterioration to gratify metabolic requirements of the body.[33] Polyunsaturated fatty acids reform blood lipid profiles by modulating the formation and oxidation of saturated fatty acids and monounsaturated fatty acids.[34] Phytol and Phytol acetate were anticipated as effective lipid metabolism regulators with Pa values 0.828 and 0.94, respectively. Phytol is a diterpene alcohol and serves as primary constituent for synthesis of phytanic acid. Phytanic acid regulates lipid metabolism by reviving of peroxisome proliferator-activated receptors.[35] Pa value for antihypercholestrolemic activity of β sitosterol was found to 0.960. β-sitosterol prevents β-amyloid protein release induced due to elevated cholesterol levels by stimulating membrane cholesterol homeostasis.[36,37] Alpha amyrin exerts hepatoprotective action (Pa = 0.926) by attenuating oxidative stress and toxic metabolite formation.[38] Amyrin esters are known to promote cell necrosis in HL-60 leukemia cells through apoptosis.[39] This is in accordance with Pa = 0.911 of alpha amyrin for apoptosis agonist activity. Lupeol was evaluated as effective antineopolastic (Pa = 0.950) and hepatoprotective (Pa = 0.907) entities. Triterpenoids are optimistic antineopolastic agents due to potential to retard tumor escalation, cell cycle succession, and stimulate apoptosis of tumor cells.[40] Lupeol possesses safeguarding effect against hepatotoxicity induced by carbon tetrachloride, paracetamol, and 7 and 12-dimethylbenz (a) anthracene.[41-43]

Tables 1 and 2 represents bioactive phytoconstituents present in female and male date palm leaves extracts respectively. Table 3 represents P_a and P_i values of different biological activity of phytoconstituents as evaluated using PASS online.

Conclusion

The present study intended to identify bioactive phytochemicals present in the extracts of male and female date palm leaves. *In silico* activity prediction of these phytoconstituents may serve as basis for further *in vivo* and *in vitro* analysis of extracts. The result of *in silico* activity prediction reveals the presence of phytochemicals with potential therapeutic applications such as anticancer, antineopolastic, hepatoprotectant, anti-diabetic, and lipid metabolism regulators. However, detailed further study is required for determination of the most potent bioactive phytoconstituent and their mechanism of action.

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